Radical Scavenging Activities, Total Reducing Power, Total Phenolic and Flavonoids Contents of Four Common Vegetables

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ABSTRACT

Aim: This study aimed at evaluating the antioxidant activities of extracts from four vegetables namely: *Beta vulgaris*, *Raphanus sativus*, and two varieties (red and green) of *Brassica oleracea*.

Method: The antioxidant properties of these extracts were assessed using five different methods including 2,2-diphenyl 1-picrylhydrazyl (DPPH), Nitrogen Oxide (NO), total reducing power, total phenolic and flavonoids content.

Results: All the four vegetable extracts showed free radical-scavenging activity against DPPH[•] with RSa50 (Radical scavenging activity 50) ranging between 129.77 and 323.64 µg/ml, and inhibitory activity against NO radical (RSa50 ranging from 1454.52 to 4479.97 µg/ml). The four vegetable extracts also showed total reducing powers ranging between 2.41 and 9.37 AAE (mg ascorbic acid equivalents per gram of dried extract). These antioxidant activities can be justified by the presence of different antioxidant compounds like total phenol contents which were present in all studied vegetable extracts with quantities varying between 4.37 and 11.83 GAE (mg of garlic acid equivalents per gram of dried extract) of dry extract, or flavonoids which were also present in all the plants with total contents ranging between 0.1 and 0.25 RE (rutin equivalents per gram of dried extract).

Conclusion: The different antioxidant activities demonstrated in this study provide scientific evidence that some vegetables commonly consumed in Cameroon including *B. oleracea*, *R. sativus* and *B. vulgaris* can serve as a dietary supplement or in preventive medicine in the management of oxidative stress and associated pathologies.

Keywords: Antioxidant, oxidative stress, phytochemical contents, vegetables.

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I. INTRODUCTION

Reactive oxygen and nitrogen species which are generated during different biological processes at low or moderate concentrations have important physiological functions, including transduction of cellular signals and defense against pathogens. If these reactive species are not regulated, they attack vital biological molecules such as proteins, RNA, DNA, lipids, and carbohydrates, leading to cell death, tissue damage, and eventually to the development of chronic diseases (Djeussiet *et al.*, 2020; Okello *et al.*, 2021). The production of the reactive species is in equilibrium with natural cleansing systems against free radicals and other reactive species present in the organism. However, ROS production sometimes exceeds internal antioxidant capacity leading to oxidative stress which is responsible for attacking vital biological molecules leading to cell death, tissue damage, and eventually to the development of chronic conditions like degenerative or cardiovascular diseases (Bagatini *et al.*, 2018; Niggeweg *et al.*, 2004). In these situations, exogenous antioxidants are important to manage or to prevent.

The exogenous antioxidants are mainly derived from food and medicinal plants including fruits, vegetables, and spices which in recent years has gained great interest in their antioxidant activity. Studies suggest that vegetables having antioxidant phytochemicals have strong protective effects against major degenerative diseases including cancer, cardiovascular and neurodegenerative diseases (Block *et al.*, 1992). Vegetables contain natural antioxidants mainly made of phenolic compounds. There is an inverse relationship between vegetable intake and the occurrence of diseases (Soengas Fernández *et al.*, 2011). In addition to vitamins, a large number of minor non-nutritious phytochemicals such as, phenolic acids, flavonoids, flavones, isoflavones, anthocyanins, catechins, and iso-catechins are present in vegetables, which reduce the risk of oxidative damage caused by free radicals. Phenolic compounds are universally distributed in vegetables and herbs and many have antioxidant properties (Cheney, 1950; Robards *et al.*, 1999).

Brassica oleracea commonly known as cabbage is one of the most important vegetables grown worldwide. It belongs to the family Cruciferae. The different cultivated varieties of cabbage show great variation with respect to size, shape and color of leaves as well as size, shape, color and texture of the head. The different forms of cultivated cabbage are classified into green cabbage, red cabbage and savoy cabbage. Cabbage is used for medicinal purposes for treating headaches, gout, diarrhea and gastric ulcers (Cheney, 1950). Much research has focused on useful phytochemicals in cabbage, particularly its indole-3- carbinole (I3C), sulforaphane and indoles which helps to activate and stabilize the body's antioxidant and detoxification mechanisms that dismantle and eliminate cancer-producing substances (Brooks *et al.*, 2001).

Beta vulgaris, commonly known as beetroots, is a vegetable of the family of chenopodiaceae widely consumed as a cooked vegetable. Its juice is consumed after extraction while its natural pigments are useful in the food industry. Beetroot is a rich source of nutrients including minerals (iron, magnesium, selenium, potassium, calcium, zinc, phosphorus, sodium) and vitamins (folic acid, vitamins A and C, vitamin

B6, niacin, and biotin. It is also rich in active secondary metabolites including phenolic compounds, saponins, and betalains which may explain its pharmacological activities like being active in the management of hypertension and cardiac dysfunction, as an antioxidant and anti-inflammatory (Georgiev *et al.*, 2010). It has been found to have antitumoral (Zhang *et al.*, 2013) and hepatoprotective effects (Rabeh, 2015).

Raphanus sativus is a root vegetable of Cruciferae family widely used for its culinary and medicinal purposes. Its medicinal properties were demonstrating since the tenth century and the plant is used today with great interest as an ingredient for the production of healthy functional foods as its rich in glucosinolates and their degradation products such as isothiocyanates (Beevi *et al.*, 2012).

The present study was aimed at evaluating the antioxidant activities of the methanolic extract of four vegetables so as to provide scientific evidence for the management of oxidative stress and associated pathologies.

II. MATERIAL AND METHODS

A. Plant Extracts Preparation

In October 2011, four Cameroonian vegetables were harvested in Dschang, West Region of Cameroon. The plants were further identified at the Cameroon National Herbarium (CNH) under the voucher specimen numbers: *Brassica oleracea* Linn; 25686 HNC (green cabbage), *Brassica oleracea* L. (red cabbage), *Beta vulgaris* n° 25664/ SRFcarn (beetroot) and *Raphanus sativus* (green radish).

Preparation of plant extracts: Each sample was air-dried at room temperature (RT), chopped and pulverized using an electrical blender to obtain a fine powder. Each plant powder was macerated in methanol. The mixture was stirred daily and 48 hours later, the resulting solution was then filtered using Whatman paper N° 1 and the filtrate, concentrated, by evaporating the solvent at 65 °C using a rotatory evaporator (Buchi R-200) to obtain the extract. The process was repeated on the residues obtained above in order to maximize yield.

B. DPPH Radical-Scavenging Assay

The free radical scavenging activity of the methanolic extract was evaluated using a procedure previously described (Kute *et al.*, 2008; Noumedem *et al.*, 2013) with slight modifications. Briefly, the test samples were prepared in methanol and 100 µL of each sample was added to 900 µL of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (a 20 mg/L DPPH) methanol solution, to give final concentrations of 50, 100, 200, 400 and 800 µg/mL. Ascorbic acid was used as a standard control. The content of each preparation was mixed and incubated at room temperature in a dark cupboard. The absorbance was then monitored after 30 min and converted into a percentage of scavenging activity using the following formula:

$$\%RSA = \frac{A_{control} - A_{test\ sample}}{A_{control}} \times 10 \quad (1)$$

The experiments were carried out in triplicate and the percentages of DPPHs scavenged by the test samples were compared to that of vitamin C. These radical scavenging percentages were plotted against the logarithmic values of the

concentrations, of extract and fractions, and a linear regression curve was established in order to calculate the RSA50 values, which are the amounts of sample necessary to decrease by 50% the free radical DPPH (Yassa *et al.*, 2008).

C. Assessment of Nitric Oxide Complexation Radical

The nitric oxide was generated from sodium, and nitoprussiate was measured using the modified Greiss reagent. For instance, 1 mL of a solution of sodium nitoprussiate dissolved in phosphate buffer (phosphate buffer saline or PBS) was added to each test tube and mixed with 100 µL of solutions of extracts to obtain different concentrations (4, 16, 64, 256, and 1024 µg/mL). The mixture was then incubated at RT for 180 min. After incubation, 100 µL of modified Greiss reagent were added to each tube. Then, the absorbance was monitored at 540 nm and converted into percentage of radical-scavenging activity (%RSA) using (2) below:

$$\%RSA = \frac{A_{control} - A_{test\ sample}}{A_{control}} \times 10 \quad (2)$$

The Probit table was then used to convert %RSA into probits which were plotted against the logarithmic values of the concentrations and a linear regression curve was established to calculate the RSA50, which are the amounts of sample necessary to decrease by 50% the free radical NO. The experiment was carried out in triplicate and the percentages of NO scavenged by test samples were compared to that of rutin.

D. Determination of Total Reducing Power

The total reducing power of each plant extract was determined as followed: the reaction mixture was prepared in phosphate buffer sodium (PBS) (0.2 M, pH 6.6) with 100 µg/mL of each extract, 440 µL of potassium ferrocyanide ($[K_3Fe(CN)_6]$ 1 %) and 440 µL of trichloroacetic acid 10 %. The samples were then centrifuged for 10 min at 3000 rpm, and 680 µL of supernatant from each tube were collected and introduced into a new tube containing $FeCl_3$ (140 µL) and demineralized water (680 µL). The absorbance of these new solutions was read at 700 nm and converted into milligram per gram of ascorbic acid equivalents (AAE) of dried extract using a calibration curve of ascorbic acid.

E. Determination of Total Phenols Content

The determination of the total phenolic content of plant extracts was carried out using the technique of Folin-Ciocalteu (Singleton & Rossi, 1965). Briefly, a stock solution of Folin-Ciocalteu reagent was diluted 10 times. Then, 500 µL of this solution was added to 100 µL of extract (10 mg/mL) dissolved in DMSO 10%. After 4 min of incubation at room temperature (RT), 400 µL of a 7.5% Na_2CO_3 solution were added to the mixture and the absorbance of the final solution was read at 765 nm. The absorbance was further converted into milligram per gram of Gallic Acid equivalents (GAE) of dried extract using a calibration curve prepared using different concentrations of gallic acid (Fig. 3) used in the same manner as extracts.

F. Determination of Total Flavonoids Contents

The determination of the total flavonoid content of each plant extracts was carried out using the spectrophotometric

method of aluminum chloride ($AlCl_3$) using rutin as a standard flavonoid. Briefly, 100 µL of each plant extract (10 mg/mL) were mixed with 400 µL of MeOH, 20 µL of $AlCl_3$ 10 %, 20 µL of acetic acid (CH_3COOH , 1 M), and 560 µL of distilled water. The reaction mixture was then incubated for 4 min at RT. After the incubation period, the measure of the absorbance was done at 415 nm and converted into µg per gram of rutin equivalents (RE) of dried extract using a calibration curve (OD Vs. rutin concentration).

III. STATISTICAL ANALYSIS

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA) v. 19.0 (IBM, Armonk, NY, USA). The results of this experiment were expressed as the mean $\pm$ standard deviation and compared using the Waller-Duncan test. The results were statistically significant for p-value < 0.05.

IV. RESULTS

The antioxidant properties of extracts were assessed using five different methods. From these experiments we obtained variable results according to the extracts and tests used.

The Probit curve of the inhibition percentage as a function of the decimal logarithm of the extract concentrations made it possible to determine the RSA50 for each extract. The results of these RSA50 are presented in Tables I and II.

TABLE I: DPPH RADICAL SCAVENGING ACTIVITIES OF THE METHANOL EXTRACTS OF THIRTEEN ANTIBACTERIAL EDIBLE PLANTS

Plant extracts	Radical scavenging activities expressed as RSA ₅₀ (µg/mL)
<i>B. vulgaris</i>	175,49 $\pm$ 9,50 ^f
<i>B. oleracea</i> (red)	129,77 $\pm$ 4,17 ^{bc}
<i>B. oleracea</i> (green)	323,64 $\pm$ 27,50 ^h
<i>R. sativus</i>	165,49 $\pm$ 9,50 ^f
<i>L-ascorbic Acid</i>	1.86 $\pm$ 0.10 ^a

RSA₅₀: concentration of tested sample necessary to decrease by 50% the free radical NO. RSA₅₀: concentration of tested sample necessary to decrease by 50% the free radical DPPH

Fig. 1. NO radical scavenging activities of the methanol extracts of four vegetables.

TABLE II: NO RADICAL SCAVENGING ACTIVITIES OF THE METHANOL EXTRACTS OF FOUR VEGETABLES

Plant extracts	Radical scavenging activities expressed as RSa ₅₀ (µg/mL)
<i>B. vulgaris</i>	1454,52 ± 92,44 ^b
<i>B. oleracea</i> (red)	2253,70 ± 29,88 ^c
<i>B. oleracea</i> (green)	4479,97 ± 92,67 ^c
<i>R. sativus</i>	2761,99 ± 52,21 ^d
<i>L-ascorbic Acid</i>	157,72 ± 7,38 ^b

RSa₅₀: concentration of tested sample necessary to decrease by 50% the free radical NO. RSa₅₀: concentration of tested sample necessary to decrease by 50% the free radical DPPH

The plant extracts tested all exerted moderate scavenging activities on DPPH° free radical compared to ascorbic acid used as reference which showed an RSa₅₀ value of 1.86. *Brassica oleracea* (red) was found to be the most active extract with an RSa₅₀ of 129.77 µg/ml. In decreasing order of their free-radical scavenging activities against DPPH°, the other extracts are classified as follows: *R. sativus* (165.49), *B. vulgaris* (175.49), and *B. oleracea* (green) (323.64 µg / ml) (Table I).

The plant extracts tested all exerted concentration-dependent radical-scavenging against NO° as shown in Fig. 2. These activities are characterised by relatively high RSa₅₀ vis-à-vis the NO° radical varying considerably from one extract to another, resulting in a relatively low free-radical scavenging activity compared to ascorbic acid. The extract of *B. vulgaris* with an RSa₅₀ of 1454.52 µg/ml showed the strongest anti-free radical activity against NO°, followed by *B. oleracea* (red) and *R. sativus* with RSa₅₀ of 2253.70 µg/ml and 2761.99 µg/ml respectively (Table II).

(*) AAE : µg of Ascorbic Acid Equivalents per gram of dried extract

Fig. 2. Total reducing power of the methanol extracts of four vegetables.

(*) GAE : mg of Garlic Acid equivalents per gram of dried extract

Fig. 3. Total phenolic content of the methanolic extracts of four vegetables.

With RSa₅₀ of 4479.97 µg/ml, the extract of *B. oleracea* (green) appeared to be the lowest free-radical scavenging potential against NO°.

The total reducing power of the four plant extracts of this work was evaluated and the results presented in Fig. 2. It follows from the analysis of this fig that, the extracts exhibited a total reducing powers varying between 2.41 and 9.37 AAE. This highest reducing power was observed with the extract of *B. vulgaris*. It emerges as the most reducing extract with a reducing power almost twice as high as those of *B. oleracea* (red) and *R. sativus* which presented a total reducing powers of 5.29 AAE and 4.79 AAE while the extract of *B. oleracea* (green) showed the lowest reducing power of 2.41 AAE.

The total phenolic content of the four plant extracts from this study was evaluated using the Folin ciocalteu method and the results presented in Fig. 3. It appears from this table that the extracts presented phenolic contents varying from one extract to another with values between 4.37 and 11.83 mg of Garlic Acid Equivalents per gram of dried extract (GAE) of dry extract. *Brassica oleracea* (green) extract had the highest phenolic content (11.83 GAE) while *B. oleracea* (red) extract had the least phenolic content (4.37 GAE). The phenolic content of *B. oleracea* (green) was followed by that of *B. vulgaris* (9.28 GAE) and of *R. sativus* (6.17 GAE).

Fig. 4 shows the flavonoid content of the four plant extracts studied in this work. It appears flavonoids are present in the four vegetables with contents that varies from one extract to another with values. *Beta vulgaris* extract exhibited the highest flavonoid content with 0.25 RE, followed by *B. oleracea* (green) with a flavonoid content of 0.15 RE, while *B. oleracea* (red) and *R. sativus* presented the lowest contents with 0.1 RE.

(*) RE : µg of Rutin equivalents per gram of dried extract

Fig. 4. Total flavonoids content of the methanolic extracts of four vegetables.

V. DISCUSSION

The antioxidant activities of the vegetables of this study confirm the results of other studies that have reported the benefits of fruits and vegetables and provide information on this topic. Fruits and vegetables are known for their ability to protect living organisms thanks to their antioxidant properties. They are rich in antioxidants and several publications have been released on the various plants of this

study (Bidchol *et al.*, 2012; Ferreres *et al.*, 2009; Liang *et al.*, 2019; Rameshwari, 2015; Sotelo *et al.*, 2014; Yanget *et al.*, 2015). Some highlighted the concern about the richness of some of these common fruits and vegetables in antioxidants such as vitamin C, vitamin E and quercetin in the plants of this study. The various plant extracts studied in this work showed anti-free radical activity against the DPPH and NO° radicals. Antioxidant activities against DPPH radicals are due to the ability of chemical compounds to deliver electrons and/or protons. Sodium nitroprusside produces NO° radical after its decomposition at physiological pH (Djeussi *et al.*, 2020). The produced radical leads to the formation of nitrites from nitrates in aerobic conditions. The chromogen formed during the diazotization process with nitrite ions, and Grace's reagent of this study has a maximum absorption at a wavelength of 546 nm (Shajiselvin *et al.*, 2010). Antioxidant compounds contained in extracts of plants inhibited the formation of the chromogen by complexing with the NO° radical. The reducing power is usually linked to the effect of the compounds that are electron donors (Loganayaki *et al.*, 2013) and the reducing powers of vegetable extracts of this study could be explained by total phenolics and flavonoids contents (El Atki *et al.*, 2019) known as powerful antioxidants that can prevent or help in the management of chronic diseases such as cancer and cardiovascular diseases (Kasote, 2013; Randhoret *et al.*, 2004). Flavonoids are a group of phenolic compounds particularly clever and quick to transfer an electron mainly known for their antioxidant activity (Bruneton, 2020; Crozier *et al.*, 2009).

B. oleraceae antioxidant activities were already demonstrated in previous studies (Bidchol *et al.*, 2012; Ferreres *et al.*, 2009; Liang *et al.*, 2019; M & Rameshwari, K, 2015; Sotelo *et al.*, 2014; Yang *et al.*, 2015). Previous study corroborates the total reducing powers and DPPH radical scavenging activities found in this study which showed that red cabbage has a greater total reducing power and a greater radical scavenging activity than green cabbage (Liang *et al.*, 2019). But other results of the same study showed that red cabbage has a greater total phenol and flavonoid contents than green cabbage in contrary to our findings. In general, the total phenol content found in the present study was higher than that of the above-mentioned study. All these differences might be due to the different conditions as the plant of the present study was cultivated in Cameroon while the one from the previous study was cultivated in China.

Antioxidant activities of *R. sativus* was previously demonstrated (Barillari *et al.*, 2006; Beevi *et al.*, 2012; Eveline & Pasau, 2019; Goyeneche *et al.*, 2015; Kim *et al.*, 2016; Noman *et al.*, 2021; Shehzadi *et al.*, 2020; Sonam, 2019). With an RSa50 value of 165.49 µg/mL, the methanol extract of *R. sativus* showed a better free-radical scavenging activity against DPPH than the 70% ethanol extract which presented an RSa50 value of 359.7 µg/mL in a previous study (Noman *et al.*, 2021), but with a higher total phenol content of 95.8 GAE (compared to 6.17 GAE found in this study). This difference might be explained by the extracting solvent, but also by different cultivation conditions.

Antioxidant activities of *B. vulgaris* were previously demonstrated (Clifford *et al.*, 2015; Georgiev *et al.*, 2010; Rabeh, 2015). These previous studies use different methods making it difficult to compare results, but the total phenol

contents of *B. vulgaris* of this study was higher than the one found in a previous study (Ramos *et al.*, 2017) where the total content of *B. vulgaris* vary according to the cooking method but remain all lower than the 9.28 GAE obtained in this study.

VI. CONCLUSION

In this study, the assessment of antioxidant activity indicates that four vegetables namely *Beta vulgaris*, *Raphanus sativus*, and two varieties (red and green) of *Brassica oleracea* can serve as a source of management of ROS-related diseases like coronary diseases or degenerative diseases. Further investigation to better understand their mechanism of action is necessary to master their ability to control diseases that have a significant impact on life quality.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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